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THE INTERNATIONAL ARABIC JOURNAL OF ANTIMICROBIAL AGENTS ISSN 2174-9094

2016
Vol. 6 No. 3:3
doi: 10.3823/793

Occurrence of potential virulence factors and antimicrobial resistance markers in fecal *E. coli* isolates from infants

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Abstract

Objectives: *E. coli* is one of the first gram-negative organism colonizing the intestine of infants, and it's commonly causes various community-acquired and nosocomial bacterial infection in infants. This study explores the relationship between the incidence of antimicrobial resistance profile and virulence factors genes of *E. coli* colonizing the intestine of infants.

Methods: A total of 150 fecal *E. coli* isolates from infants aged less than one year, who were admitted to Pediatric Clinics at The Jordan University Hospital, Amman, Jordan, were investigated for their antimicrobial resistance profile and 11 common virulence factors using PCR.

Results: A total of 134/150 (89.3%) were multidrug resistant (MDR) to at least 3 antibiotic classes. Hospitalized infants carried significantly more MDR *E. coli* than non-hospitalized. The majority of *E. coli* isolates carried the virulence factors; aerobactin (33.3%), type1 fimbriae (27.3%), S.fimbriae (20%), followed by P.fimbriae (18%), haemolysin (14.7%), papG class II (12.0%), and papG class III (7.3%), whereas all isolates were negative for capsular antigens K1 and K5 genes, papG adhesion `Class I and Dr haemagglutinin. Hospitalized infants carried significantly more MDR isolates than nonhospitalized infants, and the type of milk feeding was not significantly associated with MDR isolates in both groups.

Conclusion: This study showed that increased presence of antimicrobial resistance markers (≥ 6) in *E. coli* isolates were significantly (P= 0.001) associated with less presence of potential virulence genes.

Key word: *E. coli* virulence factors, Antimicrobial resistance, Jordanian Infants

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ISSN 2174-9094
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Introduction

Escherichia coli is the most common facultative anaerobic bacterium colonize intestines of all humans. The organism can be frequently detected after few days in the new born baby's feces and become part of their intestinal normal microflora. Intestinal colonizing of the newborn infant with *E. coli* may originate from the maternal fecal flora, close contact with mother or nursing staff and hospital environment [1].

Fecal *E. coli* strains express frequently a broad variety of virulence factors involved in the colonization, adhesion, invasion, and survival of host defenses [2]. *E. coli* strains causing extra-intestinal infections especially cystitis, pyelonephritis or prostatitis carry a patterns of virulence genes, including commonly P fimbriae, capsules, haemolysin and siderophore aerobactin [3].

Most people have one or few biotypes of *E. coli* strains in their intestine at any time in their life, some of these strains colonize the intestine for months and years, while others found as transient strains with low colonizing capacities and disappear within some weeks [4].

Recent studies from various countries have reported increased intestinal colonization and infection of infants with extended-spectrum β-lactamase (ESBL)-producing *E. coli* [5-6]. ESBL-producing *E. coli* has been widely associated with both hospitalized and community-acquired infections worldwide [7-10].

The relationship between incidence of virulence factors and antibiotic resistance in *E. coli* is still not well studied. A study of Cooke et al. [11] found that the majority of virulence factors were equally distributed between antibiotic-susceptible and multiple-drug-resistant (MDR) *E. coli* isolates, while a study of Karami *et al.*, [12] has suggested that the presence of antibiotic resistance in *E. coli* strains might be associated with increased presence of certain virulence genes than susceptible *E. coli* strains.

The objective of this study was to investigate occurrence of antimicrobial resistance and potential virulence factor genes in fecal *E. coli* isolates of infants.

Patients and Materials

Patients. A total of 150 infants aged between 1-day to 1-year were included in this study after their admission for medical investigation at the pediatric department/The Jordan University Hospital in Amman. Of these, 98 (65.3%) were males and 52 (34.7%) were females. The age of children were categorized into 4 age groups **(Table 1).** The study has been approved by the high graduate committees of the Faculty of Medicine, the ethics committee of Jordan Hospital University, and Faculty of Graduate Studies/University of Jordan, Amman-Jordan and informed consent was obtained from mother of each infant.

Collection of fecal specimens. All collected fecal swabs from infants were cultured within one hour on MacConkey agar and incubated for 24 hr in 37°C,and all fecal samples grew *E. coli.* Five colonies that morphologically represent *E. coli*- like

Table 1 . Distribution of 134 MDR *E. coli* isolates according to the age of non-hospitalized and hospitalized infants*

Infant age group	No. (%) Non-hospitalized infants		No. (%) Hospitalized infants*	
	No	%	No	%
1-30 days	4	16	56	51.3
1-3 months	13	52	15	13.8
3-6 months	8	32	16	14.7%
6-12 months	0	0	22	20.2
Total	25/134	16.7%	109/134	81.3%**

* All were treated with antibiotics during their stay at the hospital
**P= < 0.05.

 Received 21 Oct 2016, Accepted 22 Nov 2016

growth were selected and sub-cultured on MacConkey again to obtain pure *E. coli* isolates. All *E. coli* isolates were confirmed using commercial Remel RapID ONE test (Remel INC, USA).

Antimicrobial Susceptibility Testing. This procedure was performed according to the recommendation of the Clinical Laboratory and Standards Institute (CLSI,2012) [13]. The results were interpreted also according to the guidelines of CLSI. The antimicrobials used in our study (µg/disk) were presented in **Table 2.**

***E. coli* DNA extraction.** The bacterial DNA was extracted using the G-spin™ Total DNA Extraction Kit (iNtRon, Gyeonggi-do, Korea) according to the manufacturer's protocol for isolation of bacterial DNA from biological fluids. All DNA preparations were tested by spectrophotometer to ensure a sufficient quantity of DNA for PCR amplification.

Multiplex PCR tests for detection potential virulence factor genes

All virulence factor genes of *E. coli* isolates were detected using three sets of multiplex PCRs as described by Nowrouzian et al., [14]. The first PCR set included primer pairs for detection Type1 fimbriae,S fimbriae, P fimbriae as well as Dr haemagglutinin. The second PCR set composed of primer pairs for detection the three *papG* alleles, and the third PCR set included primer pairs for detection haemolysin, aerobactin and the capsular antigens K1 and K5.

Concentrations and cycling conditions. The amplification was performed in a total volume of 50 µL using Fast Ready Master Mix (Promega, USA). The primer concentration used was 1 µM of each primer and a volume of 3 µL of the extracted DNA.

The following PCR conditions were set for all tests as follow; initial denaturation at 94 °C for 4 minutes, followed by 25 cycles of denaturation at 94°C for 2 minutes, annealing at 65 °C for 1minute, and extension at 72 °C for 2 minute, and a final extension at 72 °C for 3 minutes.

Gel electrophoresis. PCR products using 5 µL of each amplified sample were analyzed in agarose gel (2%) including ethidium bromide. A control 100 bp DNA ladder was add for each run. The agrose gels were run for 45 minutes at 160 volts using horizontal electrophoresis apparatus, and the gels were visualized under ultraviolet light using gel documentation system (UVP, USA). A strain of *E. coli* ATCC 51446 was included as a positive control in PCR for detection of virulence factor genes, and nuclease free water was used as a negative control.

Table 2. Antimicrobial susceptibility pattern of 150 *E. coli* isolates*

Antibiotic	No. (%) Resistant *E. coli* isolates		No. (%) R-markers	Pattern of antimicrobial resistance phenotypes (No. R-markers)
Augmentin (AUG)	141	94	30(22)	AUG,NA,TS (3)
Cefuroxime (CXM)	78	52	26 (19.4)	AUG,TS, NA, NI (4)
Cotrimoxazole (TS)	78	52	20(14.9)	AUG,TS,NI,GM,CXM (5)
Nitrofuronatoin(NI)	78	52	19(14.2)	AUG,TS,CXM,NA,NI,CTX (6)
Nalidixic acid (NA)	77	51	19(14.2)	AUG,TS,CXM,NA,NI,CTX,GM(7)
Cefotaxime (CTX)	76	50	11(8.2)	AUG,TS,CXM,NA,NI,CTX,GM,CIP(8)
Gentamicin (GM)	71	47	9(6.7)	AUG,TS,CXM,NA,NI,CTX,GM,FOX,IM (9)
Ciprofloxacin(CIP)	29	19		
Imipenem (IM)	14	9.3		

*A total of 58 *E. coli* isolates were resistant to 6 and more antimicrobial agents.

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (IBM -SPSS) version 20. Frequency and percentage were calculated for each virulence factor associated with antimicrobial resistance factors. The level of significance was set at a p value of 0.05 to test the hypothesis of no association. Fisher's exact test replaces chi-squared test when the minimum expected count is less than five.

Results

A total of 134/150 (89.3%) MDR *E. coli* isolates to 3 ≥drug classes from infant fecal samples were recorded **(Table 1).** The majority of hospitalized infants (81.3%) harbored significantly more MDR *E. coli* isolates (P**=** < 0.05) than non-hospitalized over the same study period. There was no significant difference between total incidence of MDR *E. coli* isolates among infants in association with their types of milk feeding (breast feeding, formula or mixed) **(Table 1).** The distribution of 134 MDR *E. coli* isolates with different resistance patterns is shown in **Table 2.** The distribution of virulence genes among 150 *E. coli* isolates is shown in **Table 3.** The majority of *E. coli* isolates carried different rates of virulence genes; including aerobactin (33.3%), type 1 fimbriae (27.3%), S fimbriae (20%) Followed by P fimbriae(18%), haemolysin (14.7%), pap G class II (12.0%), and papG class III (7.3%). The results indicate that virulence genes were mostly detected *in E. coli* isolates (92/150; 61.4%) with few number of antibiotic resistance markers (1-5). Increased number of antimicrobial resistance markers (≥ 6) among *E. coli* isolates (58/150; 38.6%) was associated significantly(P =0.001) with less presence of potential virulence factor genes.

Table 3. Distribution of virulence genes among 150 *E. coli* isolates*

Virulence Factors	Virulence Genes	No. (%)
P fimbriae	*papC*	27(18)
S fimbriae	*sfaD/E*	30 (20)
Type 1 fimbriae	*fimA*	41 (27.5)
papG adhesion `Class I `	*papG*	0
papG adhesion `Class II	*papG*	18 (12)
papG adhesion `Class III	*papG*	11 (7.3)
Dr haemagglutinin	*draA*	0
Capsule K1	*neuB*	0
Capsule K5	*kfiC*	0
Aerobactin	*iutA*	50 (33.3)
Haemolysin	*HlyA*	22 (14.7)

* MDR *E. coli* isolates (58) carried 6-9 antimicrobial R-markers were associated with significantly (P= 0.001) less virulence factors.

Discussion

This study has shown that multidrug resistance (MDR) *E. coli* can rapidly colonizing the intestine of the majority of Jordanian new borne infants (89.3%) within one year-old as demonstrated in their fecal samples, particularly during hospitalization.This result showed higher incidence of MDR *E. coli* isolates than previous studies carried out in Jordan on fecal *E. coli* isolates from infants and adults over the last 8-year [5,15-16], however, it is important to note her that this study included more hospitalized patients than other studies. A recent study has also demonstrated high occurrence rate of CTX-M ESBL-producing *E. coli* colonizing the intestine of Jordanian infants at the same Jordan University Hospital [5]. It has been documented that CTX-M-15 group is widely prevalent among *Enterobacteriaceae* in Arab Middle East region and mostly associated with MDR organisms [10].

Numerous studies have demonstrated that *E. coli* colonizing human intestines might spread to other body sites and causes extra-intestinal infections, es-

 Received 21 Oct 2016, Accepted 22 Nov 2016

pecially blood sepsis and meningitis in infants and urinary tract infections in all ages [17-19] Therefore, it is expected that presence of MDR among fecal *E. coli* strains in infants would be certainly complicated successful antimicrobial treatment, particularly if these are associated with ESBL- producing *E. coli* strains which are currently emerging worldwide [10,20].

E. coli expresses a variety of virulence genes which are involved in adhesion, colonization and invasion, and those *E. coli* strains which carry few virulence factor genes rarely cause extraintestinal infections, especially urinary tract infections [2,17]. The present study showed that 61% of *E. coli* fecal isolates carried at least 5 virulence genes **(Table 3).** This study has also demonstrated that potential virulence genes were distributed in various rates among all *E. coli* isolates in association with presence of 1-5 antimicrobial resistance markers, while all isolates with antimicrobial resistance markers of ≥6 were significantly (P= 0.001) carried less potential virulence genes. Few previous studies have also reported that presence of quiniolone / fluoroquinolone resistance in *E. coli* is associated with less virulence factors and less capability of these strain to cause urinary tract infection [21-24]. However, the study of Cooke *et al*. [11], investigated frequencies of virulence factors and multidrug resistance between community and nosocomial *E. coli* isolates from bloodstream and found that the majority of virulence factors were equally distributed between antibiotic-susceptible and multiple-drug-resistant isolates. In addition, the overall virulence factor score was higher for isolates from community and health care-associated origin than those of nosocomial origin. Therefore, the link between antimicrobial resistance and virulence factors in clinical *E. coli* isolates remains unclear, and depends on the interactions between the phylogenetic background of the strain and the type of resistance determinant and source of the *E. coli* strains either from clinical infection or colonization site [21].

In conclusion, this study provides new insights into the relationships between increased presence of antimicrobial resistance markers (≥6) in fecal *E. coli* isolates from infants and the less presence of important potential virulence factors which are necessary to enhance their potential extra-intestinal infections.

Conflict of Interest

There is no conflict of interest.

Acknowledgement

This study was supported with a grant No.(103/13-2014-2015) from the Deanship of Academic Research, The Jordan University, Amman, Jordan, and also thankful for the administration support of The Jordan University Hospital in Amman.

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